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Parts III-IV

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PARTS III-IV]

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FURTHER OBSERVATIONS ON THE MIRACIDIUM  
OF *SCHISTOSOMA SPINDALIS*  
MONTGOMERY, 1906

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Received on July 21, 1955

(Communicated by Dr. H. R. Mehra)

INTRODUCTION

IN view of the fact that several aspects of the miracidium of *Schistosoma spindalis* Montgomery, 1906 have not been adequately described, this opportunity has been taken to present some additional observations on its biology and morphology. Rao (1934) published a paper entitled "A comparative study of *Schistosoma spindalis* Montgomery, 1906 and *Schistosoma nasalis* n. sp." In this paper he also described the miracidia of these 2 species of schistosomes and compared their characters in a tabular form. The description of the miracidium of *S. spindalis* as given by Rao (1934) is brief, and in certain respects inaccurate. Further study permits a more adequate description of this species which may aid in comparing it with other schistosome miracidia.

MATERIAL AND METHOD

Eleven snails of the species *Indoplanorbis exustus* infested with cercariae of *S. spindalis* were obtained through the courtesy of Dr. Rao, Parasitologist, Veterinary College, Bombay, in December 1951. A kid and a lamb

both young and clean were infected by cutaneous routes with large number of cercariæ from the infested snails. The fæces of the lamb and kid became positive for the eggs of the parasite 75 and 60 days respectively after infection.

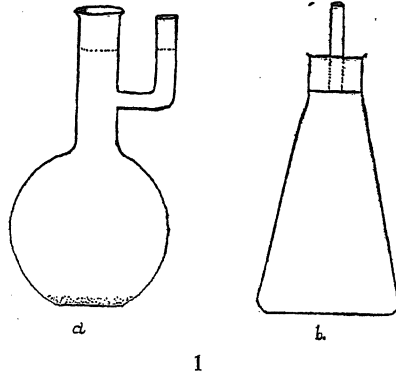
The eggs of the parasite were hatched according to the methods used in the study of the miracidium of *S. indicum* by me in 1950. Fæces containing large number of eggs after washing several times with water so as to remove acidity as far as possible were put in the hatching flasks as used by McMullen and Beaver (1945) and Stunkard (1946). The miracidia were studied alive unstained or stained with neutral red. For the study of the epidermal plates, miracidia were fixed in 1% silver nitrate solution and exposed to sunlight for about 20 minutes after which they were thoroughly washed with distilled water and mounted in glycerine.

#### DESCRIPTION

*Hatching.*—In the freshly deposited fæces, the mature ovum shows a miracidium inside it and it appears that in the majority of eggs the anterior end of the miracidium is directed towards the unspined end of the shell. Inside the egg shell which is quite transparent, the miracidium surrounded by a vitelline membrane occupies only the central area and the space between the egg shell and the vitelline membrane contains a fluid filled with granules which is probably a by-product of excretions of the larva as observed in the case of *S. indicum*. The eggs swell immediately on addition of water and the hatching is caused both by the increased osmotic pressure due to absorption of water and by the activity of the miracidium. The miracidium emerges from the egg shell through a transverse slit formed in the egg shell at about the middle. The slit is formed on account of violent rotations of the miracidium in the capsules. The important factors which govern the hatching of eggs are suitable temperature, cleanness of fæcal matter and pH range of water. The eggs of *S. spindalis* usually hatch when the temperature of water in the flasks varies from 25–30° C. and pH ranges from 6.0–7.6, a condition very similar to that observed in the case of *S. indicum* by me in 1950. Under the above suitable conditions the eggs hatch completely within 20–45 minutes, and the miracidia accumulate in large numbers in the tubes of the 2 types of flasks (Fig. 1).

*Form and Size.*—The form and size of the miracidium varies greatly on account of contraction and extension but in the normal state the body is more elongated, narrow, ciliated and bilaterally symmetrical with a tapering anterior and a narrow somewhat conical posterior end. The maximum width of body lies in level with the lateral processes. The anterior papilla is non-ciliated and capable of protraction and retraction to a great extent.

The eye spots are absent. The miracidium is somewhat larger in size than that of *S. indicum* and *S. nasalis*. Ten specimens stained with neutral red when measured after they had become motionless, have body 0.15 mm.–0.18 mm. (average 0.168 mm.) long and 0.045–0.06 mm. (average 0.055 mm.) broad.

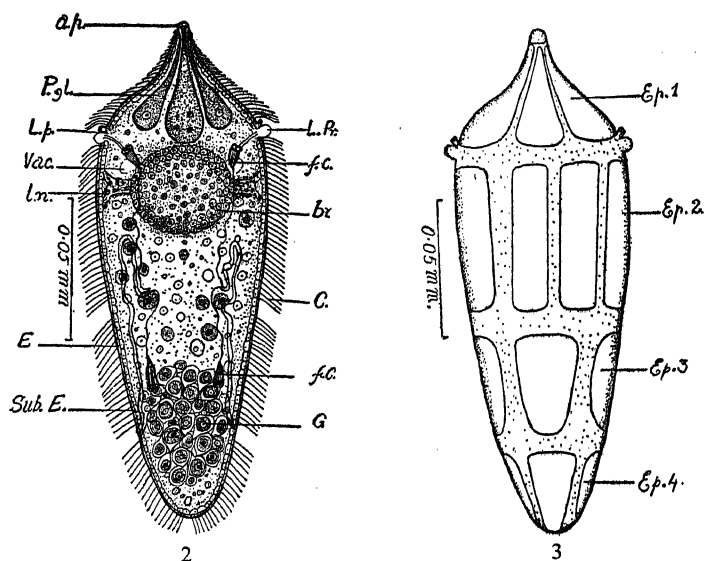


TEXT-FIG. 1 *a, b.* (*a*) Hatching flask devised by McMullen and Beaver (1945); (*b*) Hatching flask devised by Stunkard (1946).

**Movements.**—The miracidia are positively phototropic and move towards light. They swim rapidly with the aid of their cilia much in the same way as observed in the case of *S. indicum*. They move upwards and downwards when observed in a cylindrical vessel and also have a tendency to assemble near the surface of water. While swimming the larva revolves on its long axis and the course taken by it is generally a straight one.

**Longevity.**—Freshly hatched miracidia when put in distilled water, tap water and stream water all well aerated were found to live for 3–4 hours; 10–15 hours; and 24–36 hours in them respectively.

**Epidermal plates.**—The number and arrangement of ciliated epidermal plates are similar to that of other species of schistosome miracidia. There are 21 plates arranged in 4 transverse tiers (Fig. 2). The first row contains 6, the second 8, the third 4 and the fourth 3. The cilia covering the posterior tiers are much more longer than those covering the anterior tiers and their length varies from 0.006–0.012 mm. The spaces between the plates represent the portions of the sub-epithelium obviously without cilia. The plates of the first row are triangular and extend from the base of the apical papilla to the level of the lateral processes. The plates of the second row are rectangular, narrow, elongated with rounded corners and extend upto the posterior half of the body. The cells of the third row are nearly equal in length and breadth. The 3 remaining plates of the fourth tier are triangular with



TEXT-FIGS. 2-3. Fig. 2. Miracidium showing details of structure. Fig. 3. Miracidium showing arrangement of ciliated epidermal plates.

All drawings accompanied by a reference line were made with the aid of a camera lucida and details filled in at the same magnification.

*a.p.*, Apical papilla; *br.*, brain; *cd.*, collecting duct; *ct.*, collecting tubule; *ep.*, epidermal plates; *exp.*, excretory pore; *f.c.*, flame cell; *gc.*, germ cells; *gs.*, gut sac; *l.pap.*, lateral papillae; *Lp.*, lateral process; *l.n.*, lateral nerves; *P.gl.*, penetration glands; *se.*, subepithelium; *vc.*, vacuoles.

their apices surrounding the posterior tip. Rao (1934) did not describe the epidermal plates in this species but what he observed was a girdle of groups of cilia running right round the body. This girdle, he observed to be formed of 10-12 in the miracidium of *S. nasalis* and 6-8 in that of *S. spin-dalis*. I have not been able to observe such a girdle either in this species or in *S. indicum*. It appears that so-called girdles have been mistaken for cilia arising from the epidermal plates.

*Sub-epithelium*.—It consists of small flattened cells all round the body just beneath the epidermal plates. In structure, it is more or less similar to that observed in the miracidium of *S. indicum*. The longitudinal rows of cells as found in the miracidium of *S. douthittii* Price (1931) and *S. parvus* Wall (1941) lying inside the sub-epithelium were not observed in this species. The longitudinal and transverse muscle cells beneath the sub-epithelium as reported in the miracidia of *S. mansoni* Faust and Hoffmann (1934) are also absent in this species.

*Primitive gut*.—It is more or less a sac like structure filled with coarse granules. The apical papilla which is placed at the extreme anterior tip

opens into the gut. The gut is devoid of nuclei as reported in the case of *S. japonicum* Tang (1938), *S. mansonii* Faust and Hoffman (1934) and *S. indicum* Singh (1950) and thus resembles more the gut of *S. douthittii* Price (1931). Rao (1934) also observed the gut filled with coarse granules in this species.

*Penetration glands.*—Rao (1934) has referred to these glands as anterior or lateral secretory glands. The secretions of these glands help the miracidium to bore its way through the body of the molluscs and, therefore, it is more appropriate to call them as penetration glands. The penetration glands in this species are unicellular structures, one on each side of the primitive gut, which is more or less a broad, non-cellular coarsely granular structure. The basal portion of each gland is pear-shaped while the anterior part narrows to form the duct which opens at the base of the apical papilla. Each gland contains coarsely granular cytoplasm and a clear nucleus with a distinct nucleolus. The glands are somewhat shorter than the primitive gut as already observed by Rao (1934).

*Lateral processes.*—The miracidium of *S. spindalis* has a pair of small knob-like lateral processes and closely in front of each of them a papilla situated in the space between the first and second row of epidermal plates (Fig. 2). The so-called peg-like papilla on each shoulder by Rao (1934) in fact corresponds to each of the lateral processes and not to the lateral papillæ which are definitely separate structures. Rao (1934) reported opening of ducts on the so-called lateral papillæ by him from a group of small secretory cells with fine basophilic granules situated behind the gut. During the present studies no such glands were ever observed by me. Apparently a fine nerve-like structure simulating a duct can be easily traced from each lateral process inwardly upto the level of the brain where it becomes indistinct due to the presence of a flame cell and vacuoles. As in the case of the miracidium of *S. indicum* so also in this species I did not observe any extrusion of substances from these duct-like structures as has been reported by Cort (1919), Faust and Meleny (1924), and Wall (1941) in other species of miracidia. Tang (1938) and Van Haitsma (1931) also did not observe extrusion of substances in the miracidium of *S. japonicum* and *Diplostomum flexicaudum* respectively.

*Lateral papillæ.*—These structures are characteristic of Strigeid, Clino-stome, Spirorchid and Sohistosome miracidia in general and have been reported by many authors. Rao (1934) did not describe these structures. The miracidium of *S. spindalis* has also a pair of lateral papillæ on each side in front of the lateral processes much in the same manner as described for the miracidium of *S. indicum* by me in 1950.



*Nervous system.*—Rao (1934) described a fairly large circular looking object consisting of small secretory cells with fine basophilic granules behind the gut. In this group he found a pair placed close together and the ducts of each pair of groups opening on a peg-like papilla on each shoulder of the miracidium. Surrounding these 2 groups of secretory cells he found the nerve tissue with a few short linear prolongations proceeding posteriorly into the parenchymatous tissue. My observations show that the nerve mass or brain rounded in shape with 2 lateral nerves is situated in the anterior region of body in the median line just behind the gut. It measures about 0.03 mm. in diameter and contains principally of very fine nerve fibres and cells the latter accumulated more around the periphery than in the centre. The nerve or brain has been described by many workers under different names. Price (1931), Ameel, Van der Woude and Cort (1953) call it as 'central nerve mass', Faust (1949) refers the structure to be 'posterior penetrating gland'. Recently Chu and Cutress (1954) have found the structure to be a large sac-like organ lacking many characteristics of either a gland or a ganglion in the miracidium of *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953.

*Excretory system.*—The excretory system is characteristic of schistosomes and also resembles closely that of strigeids, clinostomes and spirorchids. There are 2 pairs of flame cells in the body, the anterior pair lying lateral to the nerve mass and the posterior pair located in level with the half of the third row of epidermal plates above the nephridiopore. The capillaries from these flame cells are less convoluted and join in level with the space left inbetween the second and third row of epidermal plates to form the common collecting ducts. The main ducts are also not convoluted to that extent as in *S. indicum*. The excretory pore is situated laterally in the non-ciliated space between the third and fourth tiers of epidermal plates.

*Germ cells.*—The germ cells in the miracidium of *S. spindalis* are larger, more or less quadrangular bodies according to Rao (1934). The present observations show that the germ cells are quite larger rounded and scattered in the posterior region as found in the miracidium of *S. indicum*. They are not concentrated in the form of a germ ball, the individual cells being attached to the sub-epithelial layer by fibre-like extensions of their bodies which give them more or less a quadrangular shape.

#### SUMMARY AND CONCLUSIONS

A few morphological details of the miracidium of *S. spindalis* Montgomery, 1906 not previously reported are presented. The eggs of the parasite

are passed out with the fæces of the host and hatch at a temperature of 25–30° C., a pH range of 6–7·6 and in light than in darkness. The miracidia free themselves from the capsules by rotating violently until a transverse slit is formed in the egg shell at about the middle. They are negatively geotropic and positively phototropic.

Though the miracidium of *S. spindalis* resembles other human and animal schistosome miracidia in having 21 ciliated epidermal plates, a pair of penetration glands, a sac-like gut a rounded mass of nervous tissue, the brain, 2 pairs of flame cells and many germ balls scattered in the posterior region of body, yet it can be distinguished from them in having a little longer, narrow body, 1 pair of lateral papillæ, penetration glands a little shorter than the gut and a few other minor details.

#### ACKNOWLEDGEMENTS

The author is grateful to Dr. H. R. Mehra, M.Sc., Ph.D. (Cantab.), F.N.I., F.N.A.Sc., Professor of Zoology, University of Allahabad, for his keen interest and guidance in this work. Thanks are also due to Dr. Rao, Parasitologist, Veterinary College, Bombay, for providing live snails infested with the cercariæ of *Schistosoma spindalis* for experimental purposes. The author feels indebtedness to the Government of India Ministry of Education, New Delhi, for awarding the Senior Research Fellowship for this work under Prof. H. R. Mehra.

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# FURTHER STUDIES ON STRIGEID CERCARIAE FROM ALLAHABAD

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Received on July 21, 1955

[Communicated by Prof. H. R. Mehra, M.Sc., Ph.D. (Cantab.), F.N.I., F.A.Sc.]

## INTRODUCTION

THE purpose of this paper is to present some observations on two new species of strigeid cercariae, *Cercaria kaniharii*, n. sp. parasitic in the snail *Indoplanorbis exustus* and *Cercaria rithaianensis*, n. sp. found in the snail *Lymnaea luteola* f. *australis*. The former is pharyngeal, longifurcate, monostomate, with pigmented eye spots and closely resembles the cercariae of the sub-subfamily Crassiphialini Dubois, 1936. The latter is a pharyngeal, longifurcate, distome cercariae with unpigmented eye spots and penetrates quickly tadpoles of *Bufo melanostictus* and develops in Diplostomulum type of metacercaria. The two forms have been described in detail and their relationships discussed.

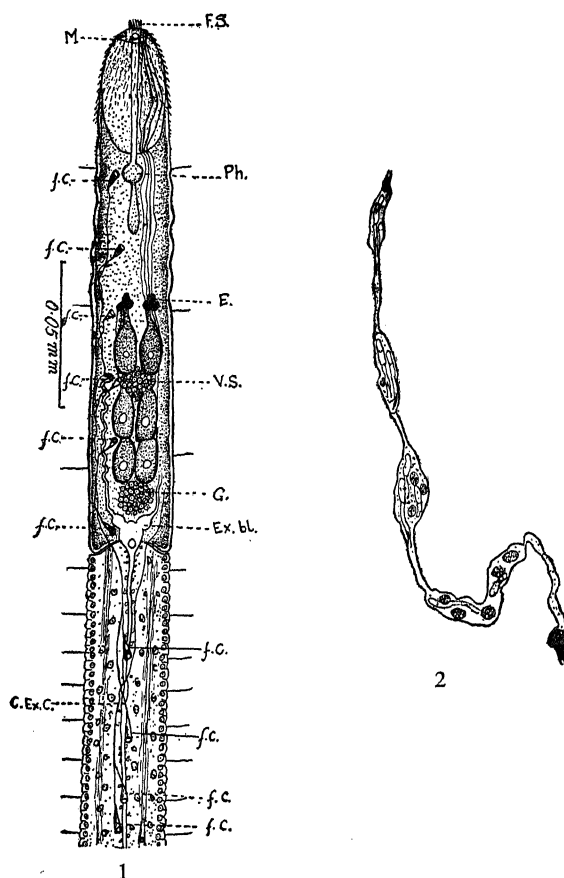
*Cercaria kaniharii* n. sp.

(Figs. 1-2)

A collection of 96 *Indoplanorbis exustus* from a pond near village Ahimanpur had two specimens infected with this species of cercaria. The cercariae after their emergence from the snail host hang in water in a manner similar to that described for other cercariae of the sub-subfamily Crassiphialini by me in 1953.<sup>1</sup> Its anterior end is bent ventrad to form a characteristic hook, the tail remains well extended and the furcae diverging at an angle of about 90° to the tail-stem. The cercaria hangs fairly motionless for some time when the furcae suddenly flip and the worm shoots upward in water to regain its original position. The cercaria moves forward with the tail in advance dragging the body behind. Characteristic annulations about seven in number in the anterior half of body are formed when the worm elongates its body, but they disappear when the body is contracted.

Normally the tail and furcae are of the same length, the body being slightly smaller. Under cover glass pressure the body, tail and furcae measure 0.195 mm., 0.26 mm. and 0.247 mm. in length respectively. The maximum width of body is 0.03 mm. and that of the tail-stem is 0.04 mm.

The furcæ measure 0·02 mm. wide at their bases. Ten specimens fixed in 10% hot formalin when measured have body 0·234–0·314 mm. (average 0·282 mm.) long and 0·026–0·033 mm. (average 0·027 mm.) broad. The tail-stem measures 0·33–0·38 mm. (average 0·035 mm.) long and its width is equal to that of the body. The furcæ are 0·33–0·36 mm. (average 0·35 mm.) long and have an uniform width of 0·02 mm.



TEXT-FIGS. 1–2. Fig. 1. *Cercaria kaniharui*, n.sp., showing details of body and tail. Fig. 2. A portion of sporocyst of *C. kaniharui*, n.sp.

B., Birth pore; C.Ex.C., Caudal excretory canal; E., Eyes; Ex. bl., Excretory bladder; F.S., Forwardly pointing spines; f.c., Flame cells; G., Genital primordium; M., Mouth; Oe., Oesophagus; Ph., Pharynx; V.S., Ventral sucker.

The body is elongated with a round, narrow anterior end and a sub-quadrate posterior end. The elongate, relatively large terminal anterior penetration organ empties into a very narrow prepharynx and a tiny globular pharynx (Fig. 2). The rhabdocele gut represents the development of the

digestive system beyond the pharynx. The anterior penetration organ measures 0·05–0·06 mm. in length and has an uniform width of 0·026 mm. in preserved specimens. A true ventral sucker is absent and represented by a mass of small rounded cells separating the anterior pair of penetration glands from the two posterior pairs. The six large clearly nucleated penetration glands lie in the posterior region of body, the three glands of each side being arranged in a linear fashion and taper into ducts which run anteriorly to enter the anterior penetration organ where they become greatly swollen. They open at the anterior end where they are associated with large spines located dorsal to the opening of the mouth in a circumoral spineless area.

A mass of deeply staining cells with neutral red, the germinal mass is posterior to the penetration glands and anterior to the excretory bladder. Many small nuclei are scattered over the body, and a few long sensory hairs on each side.

The tail-stem is attached terminally and bears sensory hairs about 15 in number on each side. Caudal bodies are absent but surface nuclei are distributed regularly along either side of the tail-stem and also through the furcæ. Very fine annulations due to contraction of circular muscles are present in the tail and also extend from the bases to the tips of the furcæ. Loose muscle bands extend throughout the length of the tail and terminate in furcæ. The furcæ bear minute spines at the bases and end in sharp points.

Small thick spines cover the anterior end of the oral apparatus but thin out rapidly posteriorly reaching only to a point about mid-way between the anterior organ and the eye spots. The small pigmented eyes are mid-way between the oral organ and the posterior end of the body.

A small excretory bladder lies at the posterior end of body and is constricted in its middle so as to give the appearance of a double bladder. A small 'Island of Cort' is present. The main excretory ducts of body originate as elongations of the antero-lateral arms of the bladder. Anteriorly each of the main collecting ducts of body divides into an anterior and a posterior branch. The anterior branch ends in three capillaries each with its flame cell and the branches of the posterior duct end in five flame cells three of which are inside the body and two in the tail (Fig. 2). Extending posteriorly from the bladder is a single median tubule which runs along the longitudinal axis of the tail-stem and divides near the fork. Each branch of this tubule extends through one half the distance of the furca before opening at its lateral border.

The daughter sporocysts of *C. kaniharii* n. sp. are yellow thread-like structures which are so tangled in the liver mass of the snail that individuals are not easy to separate. Many parts of sporocysts were studied and they were found to be filled with germ balls and cercariæ in different stages of development. The anterior end of the sporocyst contains a birth pore and the walls are constricted at places without germ balls.

#### DISCUSSION

*Cercaria kaniharii* n. sp. is the only species of pharyngeal, longifurcate, monostome cercaria which resembles *C. multicellulata* Miller, 1926, *Cercaria* of *Posthodiplostomum minimum* (Mac Callum, 1921) Doubois, 1936, *C. bessiae* Cort and Brooks, 1928, the larval stage of *Uvulifer ambloplitis* (Hughes, 1927) Doubis, 1936 and *C. physæ* Cort and Brooks, 1928. All these above species are monostomate, in having a rudimentary ventral sucker, six penetration glands, long hairs or setæ on body and tail-stem, and similar pattern of excretory system. The eyes in the new species are exactly similar to those of *C. multicellulata*, Miller (1926), *C. physæ* Cort and Brooks (1928) and *C. Posthodiplostomum* Miller (1954) but the caudal bodies which are characteristic of the three species are absent in the new species, and thus resembles *C. bessiae* in the nature of the tail-stem. The body spines, digestive system of *C. kaniharii*, n. sp. are similar to those of *C. bessiae* and *C. physæ* whereas in *C. multicellulata* and *C. posthodiplostomum minimum* the body spines extend further back and the digestive system shows no trace of œsophagus and intestinal cæca. The forwardly pointing spines in the circumoral spineless area are absent in *C. multicellulata* but present in the other species.

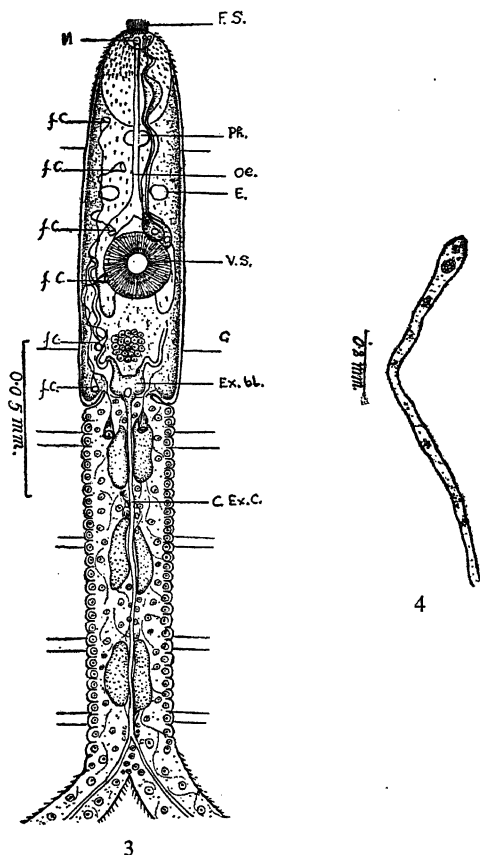
The two species *C. bessiae* and *C. kaniharii*, n. sp., though differ from one another in the nature of the eye spots yet are identical as regards the pattern of the excretory system and the number of the flame cells, forwardly pointing spines, swimming and resting habits, digestive system, tail-stem and many other characters so much so that the new form should be included in the Crassiphalini Group of Strigeid cercariæ.

#### *Cercaria rithaianensis*, n. sp.

(Figs. 3-4)

*Cercaria rithaianensis*, n. sp. is a small form obtained from two specimens of *Lymnæa luteola* f. *australis* out of 52 collected from a pond near village Rithaian about 15 miles east of Allahabad in April 1953. After escaping from the host, cercariæ keep up an almost constant activity swimming forth and back in water by vibrating the body and tail. Rarely they

come to rest in water with the body hanging downwards, tailstem turned upwards and furcæ diverged at an angle of about  $60^\circ$  to the tail-stem. Under cover glass pressure cercariae move forward by means of the anterior organ and the ventral sucker.



TEXT-FIGS. 3-4. Fig. 3. *Cercaria rithaianensis*, n. sp., showing details of body and tail. Fig. 4. Sporocyst of *C. rithaianensis*, n. sp.

The body is small, much granular, flexible extending upto 0.24 mm. in length. When contracted it becomes almost globular measuring  $0.18 \times 0.06$  mm. Ten specimens killed in 10% hot formalin have average body size,  $0.142 \times 0.039$  mm., tail-stem  $0.166 \times 0.036$  mm. and furcæ  $0.162 \times 0.016$  mm. The smallest specimen when measured gave body  $0.138 \times 0.41$  mm., tail-stem  $0.161 \times 0.032$  mm. and furcæ  $0.156 \times 0.018$  mm. The largest specimen had a body size of  $0.147 \times 0.0414$  mm.; tail-stem  $0.17 \times 0.036$  mm. and furcæ  $0.17 \times 0.013$  mm.



The anterior organ is oval and measures 0.07–0.08 mm. in length and 0.04–0.06 mm. in breadth. The ventral sucker is large, muscular, circular in outline and measures 0.03–0.04 mm. in diameter. The surface is provided with three rows of spines. The spination of body is characteristic and upto half the level of the anterior penetration organ the spines are thick but gradually thin out posteriorly so that at the level of the ventral sucker there are practically no spines on the body. The forwardly pointing spines present in a circumoral spineless area at the extreme tip of the snout are dorsal to the opening of the mouth and are 10–12 in number.

The tail-stem is long, cylindrical usually with three pairs of caudal bodies and lateral rows of nuclei. Long sensory hairs eight in number on each side are arranged in groups of two. The furcæ are about equal in length to that of the tail-stem, leaf-like and end in sharp points. They are much more transparent than either the body or tail-stem, and have few scattered spines at their bases.

The penetration glands are four in number, in front of the ventral sucker and smaller in proportion to the body size and coarsely granular. The ducts of these glands open on either side of the oral opening in the circumoral spineless area. A pair of small rounded unpigmented eye spots lies in front of the penetration glands. The oral opening is lined by a cuticular thickening. Prepharynx is short and the pharynx is a small globular and distinct structure. The intestinal cæca have enlarged terminal portions which end in level to the genital primordium.

The excretory system is characteristic of strigeata. The bladder is a small vesicle lying at the junction of the body and the tail-stem. It has an 'Island of Cort' from where the caudal excretory duct arises and runs back in the tail-stem. There are six pairs of flame cells in the body and a pair at the base of the tail-stem. The genital primordium is represented by a mass of small rounded cells in front of the bladder and is readily stained with neutral red.

The sporocysts of *C. rithaianensis*, n. sp. are long thread-like structures about 3 mm. in length. The walls are constricted in between the masses of germ balls. They perform worm-like movements in the living condition. The anterior end is produced into a snout which bears a birth pore guarded by two lips.

The cercariæ were found to penetrate actively tadpoles of *Bufo melanostictus*. They are not attracted by the tadpoles but by chance they come in contact and attach by their suckers, creep about, and finally penetrate the skin. During the act of penetration the cercariæ bend actively several times

at the junction of the body and the tail and finally throw off the tail. After penetration, the larvæ migrate to the sides of the vertebral column, in the body cavity, in the head region and also in the tail. Within few minutes all these regions become practically crowded with moving cercarial bodies. The larvæ remained unencysted for about 6 days. Examinations of 8 tadpoles on the 12th day revealed that further development of the larvæ had been arrested. Of the remaining eight tadpoles examined on the 18th day one had two larvæ in the body cavity. On examination they showed all the characteristics of a *Diplostomulum* type of metacercaria. Probably on account of high temperature and unnatural conditions in the laboratory in the month of April these experiments could not succeed. Further on account of the non-availability of more infected snails and tadpoles, the experiments had to be abandoned.

#### DISCUSSION

There are more than 17 different species of strigeid cercariae having four penetration glands in front of the ventral sucker. Of these *C. tenuis* Miller, 1923; *C. indica* XXII Sewell, 1922; *C. letifera* Fuhrmann (Dubois, 1929) and *C. bajkovi* McLeod, 1934 resemble *Cercaria rithaianensis*, n. sp. in having definite caudal bodies in the tail and without transverse excretory commissure in the body but differ in many other features.

*Cercaria marcianæ* La Rue, 1917 (Cort and Brooks, 1928), *C. indica* Sewell, 1922, *C. anuri* Singh, 1951 have no bladder commissure in body, caudal bodies in the tail and they differ in many respects from the new form. *Cercaria rithaianensis*, n. sp., though resembles *C. ranæ* Cort and Brackett, 1938; *C. stoni* Brooks, 1943; *C. stephensi* Brooks, 1943 in many of its morphological features yet differs from them specially in the number of flame cells, caudal bodies, spination and many other characters.

Strigeid cercariae without caudal bodies having two pairs of antero-lateral penetration glands and excretory commissure in body to which the new form resembles are: *C. douglassi* Cort and Brooks, 1928, the larval form of *C. flabelliformis* Faust, 1917; *C. sanjuanensis* Miller, 1927; *C. Cotylurus cornutus*, i.e., *Cercaria* A Szidat, 1924; *Cercaria fissicauda* La valette, 1855 (Brown, 1926) and *C. leplei* Brooks, 1943. All these forms differ from *C. rithaianensis*, n. sp. in having two pairs of flame cells in the tail-stem and many other characters.

*C. rithaianensis*, n. sp. comes very close to *Cercaria Neodiplostomum lucidum* La Rue and Bosma, 1927 (West, 1935); *C. Pharyngostomum cordatum* Wallace, 1939; *C. Alaria mustelæ* Bosma, 1934; *C. Alaria intermedia* Odlaug,

1940; *C. sincera* Olivier, 1941 and *C. fibricola texensis* Chanler, 1942 in having four penetration glands and no excretory commissure in body, but differs from them in having caudal bodies and in the number of flame cells.

#### ACKNOWLEDGEMENTS

The author is grateful to Dr. H. R. Mehra, Professor of Zoology, Allahabad University, for his keen interest and guidance in this work. Thanks are also due to Government of India, Ministry of Education, for granting me a Senior Research Fellowship for this work under Prof. H. R. Mehra.

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# ON THE MORPHOLOGY OF THE AIR-BLADDER OF *NOTOPTERUS CHITALA* (HAMILTON)

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## 1. INTRODUCTION

ALMOST every imaginable form of activity has been attributed to the air-bladder and the variety of functions supposed to be performed by it is greater than that of any other organ possessed by any vertebrate. The most important of its functions are connected with hydrostatic activities, phonation or respiration, etc. Air-bladder is variously modified in different groups of fishes and is always correlated with the performance of its functions. In this paper the morphology of the air-bladder of *Notopterus chitala* has been described and an attempt has been made to correlate its peculiar development with the hydrostatic function of the air-bladder.

I am grateful to Dr. D. R. Bhattacharya, formerly Vice-Chancellor of Allahabad University, for his valuable guidance, help and encouragement. The paper was prepared during the tenure of a senior Research Fellowship awarded by the Government of India, to whom I wish to express my thanks.

## 2. EXTERNAL MORPHOLOGY

Externally, the air-bladder of *Notopterus chitala* is clearly divisible into three parts—anterior auditory cæcum, median abdominal part and posterior caudal cæcum (Fig. 1, *Aud.C*, *MAP*, *CC*).

The median abdominal part is more or less cylindrical and is situated in the abdominal cavity beneath the kidney and the vertebral column. This part of the air-bladder is invested by the peritoneum on the ventral surface alone. Towards the anterior end, this portion of the air-bladder swells up into a sub-spherical sac-like structure (Fig. 1, *SSS*), and is separated by the abdominal portion by means of an oblique groove which is not very deep. Anteriorly, this sac abruptly contracts and after a shallow constriction it is continued into a somewhat narrow median prolongation which is in contact with the ventral surface of the centrum of the first vertebra and the basi-occipital bone of the skull. In its turn, this prolongation divides into two narrow tubules, termed as auditory cæca (Fig. 1, *Aud.C*) each of which diverges

from its fellow and extends forwards and outwards till it reaches the outer surface of the auditory capsule where it becomes intimately connected with the enclosed membranous labyrinth of the internal ear. Dorsal aorta (Fig. 1, *DA*) can be seen going backwards at the point where the auditory cæca originate. On both the sides of the skull the branchial cavity is also prolonged forwards which, practically, takes the form of a cæcal diverticulum and ends blindly in front. Throughout its course it runs parallel to the auditory cæcum of the corresponding side. The wall of this branchial diverticulum is very thin and thus the air-bladder and its gases are more closely related to the external medium at this point than anywhere else.

The median abdominal portion (Fig. 1, *MAP*) of the air-bladder, just before the first radial element of the anal fin, divides into two caudal cæca. These cæca, at their origin, are not very wide but are, still then, twice the dimension of the abdominal portion of the air-bladder. The cæca (Fig. 1, *CC*) extend posteriorly on both the sides of the tail and are situated wholly internal to the caudal musculature of its sides, and are in immediate contact with the hæmal spines of the caudal vertebræ. Throughout the whole length of these prolongations, their ventral margins give off fourteen short but relatively wide diverticula (Fig. 1, *SD*) which form pairs with their fellows of opposite side. Each diverticulum, except the first one, which remains undivided, in its turn, subdivides into two very slender and small filiform cæca (Fig. 1, *FC*) which extend ventrally towards the base of the anal fin. It is at the point where the second and the third diverticula arise that the width of the caudal cæcum is highest. Gradually the cæcum narrows down posteriorly and ends into a small cæcum approximately in the middle of the tail.

The ductus pneumaticus (Fig. 1, *Pn.D*) is very short and is considerably wide. There is no valvular arrangement but the opening is guarded by sphinctor muscles. The duct opens in the oesophagus on the mid-dorsal line and is very near the last pair of branchial clefts. The opening of the ductus pneumaticus into the air-bladder is found a little towards the left side of the mid-ventral line and is just behind the oblique shallow constriction which separates the median abdominal part of the air-bladder with the sub-spherical sac.

### 3. INTERNAL STRUCTURE AND ITS RELATION TO THE SKELETON

The external walls of the ventral diverticula, and the dorsal and outer walls of each caudal cæca are of moderate thickness; while the walls of the filiform cæca are very thin. But in each case, however, a relatively thick tunica externa and an extremely thin tunica interna with an internal epithelial lining is present. On the other hand, the inner wall of each caudal

cæcum, together with its primary ventral diverticula, consists of only tunica interna and is in relation with the outer surface of the radial elements and hæmal spines. These inner walls are so thin that when the cavity of the air-bladder is exposed by the removal of its outer walls, it appears as if the skeletal elements project freely into the lumen of the air-bladder. The cavities of the caudal cæca, on the opposite sides of the tail, are separated by the skeletal elements and the fibrous sheet which stretches in between them. The series of ventral diverticula are located in between the successive pairs of the radial elements, that is, each diverticula fills up the space between the two contiguous pairs of radial elements which are, dorsally, attached to the hæmal spines of the two successive caudal vertebræ. Therefore, the diverticula closely agree in number with the pairs of the radial elements. The dorso-lateral margins of the median abdominal portion of the air-bladder together with the sub-spherical sac are in close relation with the transverse processes of the trunk vertebræ. The walls of the air-bladder, at the point of these connections are very thin and, in fact, are represented by tunica interna alone.

#### 4. INTERNAL DIVISION OF THE AIR-BLADDER

The cavity of the median abdominal portion of the air-bladder is subdivided into two lateral compartments by means of a vertical longitudinal septum (Fig. 2, *VLS*) which is continuous both dorsally and ventrally with the corresponding walls of the air-bladder. As the height of the bladder increases posteriorly the septum also increases in height. Thin septum is inserted into the anterior face of the hæmal arch and spine of the first caudal vertebra and the proximal portion of the first radial element of the anal fin, at the point where the median abdominal portion of the air-bladder divides into two caudal cæca. Anteriorly, this longitudinal septum shifts from the median plane to the left side and thus the left lateral chamber becomes smaller than the right. So far as the ventral portion of the septum is concerned, it ceases immediately behind and a little distance to the right of the internal aperture of the ductus pneumaticus. But at the same time, the dorsal portion of the septum extends forwards and continues in the sub-spherical sac. Here it is connected by a narrow obliquely transverse septum (Fig. 2, *OTS*) with the left lateral wall of the bladder along the line of the external oblique groove, separating the median abdominal part of the air-bladder. Due to the asymmetrical position of the longitudinal septum and the presence of an oblique transverse septum, the communication between the sub-spherical sac and the left lateral chamber is reduced to a small triangular aperture (Fig. 2, *TA*) whereas the opening of the sac into the right lateral chamber remains wide. And due to the position of the ductus pneumaticus aperture on the left side

of the median line, the pneumatic duct opens directly into the left lateral chamber and, consequently, its connection with the right compartment becomes a bit indirect; and takes place through the cavity of the sub-spherical sac, round the free anterior margin of the ventral portion of the longitudinal septum.

There are a series of small and round vacuities in the fibrous sheath between the radian elements near the dorsal wall of the caudal cæcum (Fig. 3, *DV*). They are distributed in a regular order between the successive pairs of radial elements which are attached to the vertebral hæmal spines. The cavity of each caudal cæcum does not communicate with that of its fellow at these points as had been thought of by Bridge in case of *Notopterus borneensis*. The inner wall of the caudal cæcum of both the sides remain intact in the region of these vacuities and hence no such direct communication is possible.

Towards the ventral side of the caudal cæcum also, there is present a series of oval vacuities (Fig. 3, *VV*) bigger than the former one, in the inter-radial fibrous membrane, where the membrane in question separates the closely related inner walls of the two series of ventral diverticula. As regards their positions, they correspond with the vacuities of the dorsal-series. Wherever these vacuities are found corresponding perforations are also present in the inner walls of the diverticula, and thus the cavities of both the caudal cæca become interconnected.

As in the case of other physostomous fishes, here also there is no trace of any gas-gland.

##### 5. EAR-AIR-BLADDER CONNECTION AND ITS RELATION TO THE SKULL

The skull of *Notopterus chitala* affords a fine example of the mutual adjustment of the skull form and the body form. The concave dorsal surface of the skull rises steeply towards the high hump on the back, the great size of which aids in the balance of the body when propelled by the undulations of the enormous-sized anal fin. The orbits have moved forward to the front of the head whereas the hyomandibular has retained its articulation with the back part of the lateral wall of the brain case, as such the middle part of the skull has become much elongated behind the orbits, which is more prominent in this case than in *Notopterus notopterus* (Pallas).

The dorsal surface of the skull is long and narrow and its posterior part possesses a number of deep crests which lodge the forwardly extended strips of the body muscles. The supra-occipitals are long and are separated with the frontals by means of short parietals.



Primitive isospodyl features are met with in the lateral view of the skull. Some of the primitive features are the extension of lateral teeth upto maxilla, appearance of circum-orbital plates, presence of orbito-sphenoid and the retention of a symplectic bone. Side by side there exist specializations and losses, e.g., the close connection of quadrate with preopercular by means of two serrated ridges, the reduction of opercular which is membranous around the margin and the loss of sub-opercular.

There is a vacuity covered by a membrane in the lateral wall of the auditory capsule (Ridewood's lateral cranial foramen) leading directly into the cranial cavity in the dried skull. The base of the skull is inflated. The bulla is formed by the prootic and the basioccipital at the side and by posterior end of the parasphenoid below. Behind the bony swellings, there is a ventro-lateral vacuity bound above by the opisthotic and prootic and internally by the basioccipital. This vacuity lodges the inner and upper portion of the anterior auditory cæcum of the air-bladder.

The auditory cæca terminate at the sutural junction of the basioccipital bone with the prootic. The outer-lateral wall of this cæca consists both of the tunica externa and tunica interna but dorsally and ventrally the tunica externa is lost and the cæcum becomes firmly inserted into the corresponding vacuity of the basioccipital bone. Towards the anterior termination of the cæcum the tunica externa of the outer and lateral wall also disappears.

#### 6. RELATIONSHIP OF AIR-BLADDER AND LATERAL SENSORY CANAL

It has been shown by Omarkhan (1949) that the ear in *Notopterus* is associated, on the one hand, with a modified and specialised latero-sensory canal system and on the other hand, with the anterior diverticulum of the air-bladder. Thus the two structures, the air-bladder and latero-sensory system which, morphologically and physiologically, are unrelated to each other in normal piscine anatomy by virtue of their invasion towards a common structure—the ear—may become directly interrelated with each other, as in *Gymnarchus* and *Clupea*, or indirectly interrelated with each other, as in *Notopterus*.

#### 7. DISCUSSION

One of the most important functions attributed to the air-bladder is the hydrostatic function. The theory was first put forward by Delaroche (1809), but Moreau (1876) was the first to give any experimental evidence in its favour. If the swim-bladder functions as a hydrostatic organ, the density of a fish in which it is well developed would be expected to be close to that of its environment, while that of the fish in which the bladder was small or absent should be appreciably greater.

As it is supposed to be a hydrostatic organ, its size and extent is directly correlated with its function, *e.g.*, in Cyprinoids which are found in the rivers of the plains, the bladder is large and lies free in the abdominal cavity. It is constricted in the middle to form a posterior chamber and is joined with the oesophagus through a pneumatic duct, which opens in its constricted region. But the air-bladder differs altogether in those cyprinoid species which inhabit the rapid running waters and consequently lead a ground habit life. The fishes living in mountain torrents need a solidity of the body and not buoyancy and as such the bladder undergoes considerable degeneration. Firstly this consists in gradual reduction of the two chambers and ultimately disappearance of the posterior, and secondly in the thickening of its walls and sometimes getting enclosed in bony capsules. In the genus *Psilorhynchus* found in torrents of North-East Bengal and Assam the posterior chamber is greatly reduced and the anterior is covered by a thick fibrous coat. In *Nemachilus vittatus* (Day) from the streams of Kashmir Valley, the anterior chamber is laterally flattened and covered by bony capsule while the posterior chamber is small and thick-walled. In some extreme cases of genus *Nemachilus* the posterior chamber has disappeared altogether.

In some of the air-breathing fishes we find that the air-bladder is greatly reduced and enclosed in bone, *e.g.*, in *Heteropneustes* where the long dorsal tubes replace the ventral air-bladder. Considering all this, it is evident, that the air-bladder would be unnecessary to fishes which live on the bottom. Consequently, we find that in bottom living fishes the air-bladder is wanting, having disappeared in the course of evolution, *e.g.*, in Pleuronectidæ (flat-fishes).

Likewise, in the fishes which are laterally compressed, the bladder for want of room in the narrow body-cavity, extends in the tail region. *Notopterus chitala*, as described above, is a fine example of this. Such extensions of the air-bladder in the tail are also met with in Sparidæ and Carangidæ.

#### 8. SUMMARY

The air-bladder of *Notopterus chitala* (Hamilton) is divided into anterior auditory cæcum, median abdominal part and posterior caudal cæcum. The median abdominal part is divided internally by a vertical longitudinal and an obliquely transverse septum. There are series of small rounded vacuities dorsally and bigger oval vacuities ventrally. The cavities of both the caudal cæca are interconnected through the ventral series of vacuities and not through the dorsal series as was thought of by Bridge in case of *Notopterus borneensis* (Bleeker).

## 9. REFERENCES

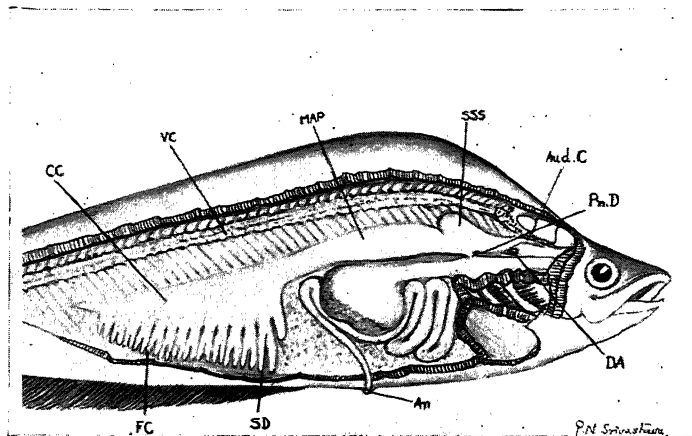
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## 10. EXPLANATION OF PLATE

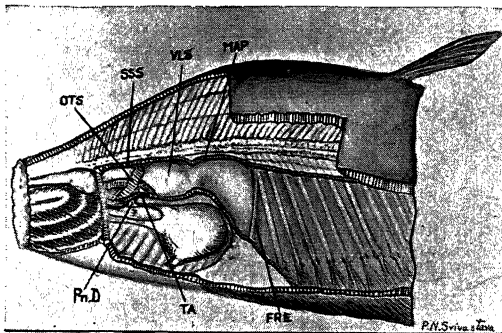
FIGS. 1-3. Fig. 1. Fish dissected laterally to show the air-bladder, anterior auditory cæcum, median abdominal portion, and the posterior caudal cæcum. Fig. 2. Lateral wall of the bladder has been removed to show the longitudinal median septum and the oblique transverse septum dividing the cavity of the bladder. Fig. 3. Outer wall of the caudal cæcum has been removed to show the dorsal and ventral series of vacuities.

## 11. ABBREVIATIONS USED

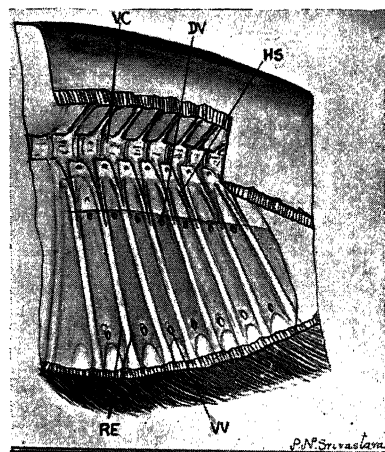
An, Anal opening; Aud.C, Auditory cæcum; CC, Caudal cæcum; DA, Dorsal aorta; DV, Dorsal vacuities; FC, Filiform cæca; FRE, First radial element; HS, Hæmal spine; MAP, Median abdominal portion; OTS, Oblique transverse septum; Pn.D, Pneumatic duct; RE, Radial element; SD, Short diverticula; SSS, Sub-spherical sac; TA, Triangular aperture; VC, Vertebral column; VLS, Vertical longitudinal septum; VV, Ventral vacuities.



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# STUDIES ON THE FEEDING HABITS AND CERTAIN ASPECTS OF THE DIGESTIVE PHYSIOLOGY OF DIFFERENT STAGES OF *PAPILIO DEMOLEUS* LINN.

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FOOD and feeding habits are interlinked with the morphology and physiology of insects. The present paper deals with the feeding habits and physiology of digestion of the different stages of *Papilio demoleus*.

## I. FEEDING HABITS

*Papilio demoleus* is a pest of the family Rutaceæ, found commonly in nurseries of *Citrus limonia* (lemon), *C. acida* (lime), *C. aurantium* (orange), *C. limetta* (sweet lime) and *C. decumana* (pomelo), but it damages the leaves of grown-up fruit-bearing trees as well. As soon as the larvæ hatch out from the eggs, laid singly, they start feeding on young leaves making holes of different sizes throughout the lamina. In extreme cases only the midribs and a few veins remain behind and the leafless infested twig appears to consist of a mass of petioles and midribs only.

The fact that this butterfly attacks the plants of a particular family Rutaceæ, rather the particular genus *Citrus*, goes to show the importance of odour in the selection of food. Field observations revealed that the incidence of larvæ was quite heavy on *C. aurantium* even when young plants of other species of *Citrus* were growing nearby. In order to confirm this observation by laboratory experiments, leaves of more or less the same size and age, of different species of *Citrus*, were put in a big petri-dish towards the periphery, and the larvæ of practically the same age were liberated in the centre so that all the leaves were almost equidistant from the larvæ. After an interval of 24 hours, orange leaves were found to have been eaten to the maximum. The preference of the larvæ for orange leaves goes to show that taste plays an important role in the selection of food and as the infestation is more common in the nurseries than on grown-up plants, it shows that the age of the host plant influences the choice. Thus odour, taste and age of the host plants,—all the 3 factors—determine the selection

of food by the larvæ, but the adult visits the flowers of *Citrus* irrespective of the age of the plant.

## II. DETERMINATION OF THE HYDROGEN-ION CONCENTRATION IN THE SALIVARY GLANDS AND IN DIFFERENT PARTS OF THE GUT

The study of H-ion concentration is an important aspect of the study of digestive physiology as different enzymes act under different H-ion concentrations. To determine H-ion concentration, paper indicator method was used as the quantity of secretions available was very small. The indicator paper was of Merck and the water used wherever necessary was conductivity water.

The larvæ and adults were dissected after starving them for 4-6 hours so as to clear the gut of the food contents to a great extent. Then salivary gland and different parts of the alimentary canal were separated, and slightly teased and brought in contact with pieces of paper indicator, first with the universal indicator paper and later with those of finer ranges. The results obtained have been tabled below:—

*pH of regions of different stages of Papilio domoleus*

Region	Stages						
	Larva	Pre-pupa	Pupa 1 day old	Pupa 2 days old	Pupa 3 days old	Late pupa	Adult
Blood	.. 6.4	6.6	6.2-6.4	6.2	6.6	6.4-6.5	6.4
Salivary gland	.. 6.8	6.8	..	..	..	..	6.2
Foregut	.. 9.5	6.8	6.4	6	6.8	6.2	6.0
Midgut	.. 8.8-9.0	7.0	6.4	6.6	6.8	6.2-6.4	6.2-6.8
Food of midgut	.. 9.3	..	..	..	..	..	..
Hindgut	.. 6.4-6.6	7.0	6.4	6.6	6.8	6.2	6.0
Excreta	.. 5.7	..	..	..	..	..	5.7-6.0

*pH of the leaves of different Host Plants of Papilio demoleus*

Insect	Host plant (leaf)	pH
<i>Papilio demoleus</i>	<i>C. limonia</i> (lemon)	5.8
	<i>C. aurantium</i> (orange)	5.9
	<i>C. acida</i> (lime)	6.0
	<i>C. limetta</i> (sweet lime)	5.4
	<i>C. decumana</i> (pomelo)	6.2

## III. QUALITATIVE ESTIMATION OF ENZYMES

*Preparation of the Extract*

Larvæ, pupæ and adults were chloroformed and dissected immediately under distilled water. Salivary glands and different parts of the alimentary canal were taken out and ground with glycerine. The microtubes were then filled with them about  $\frac{1}{3}$  of the capacity, the remaining  $\frac{2}{3}$  of the tube was filled with toluene.

*(a) Detection of the presence of amylase*

*Test 1\*.*—Two drops of the tissue suspension of salivary gland, foregut, midgut and hindgut were taken in separate microtubes while respective boiled suspensions in separate microtubes. To each of these were added 2 drops of 0.5% boiled, soluble starch solution and the rest of the tube was filled with toluene and incubated from 72–96 hours—the maximum time required for the decomposition of starch. After 96 hours potassium iodide-iodine test (to a solution of potassium iodide, iodine was added to get a solution of iodine) was performed.

*Observation.*—The test microtubes containing the salivary gland of the larva and prepupa, and of the midgut of larva, prepupa and pupa upto the age of 3 days did not turn blue, the rest turned blue (salivary glands of pupæ could not be tested).

*Inference.*—Amylase is present in the salivary gland of larva and prepupa and in the midgut of larva, prepupa and pupa upto the age of 3 days.

\* Tests for proteases were performed on the lines of Hickernell (1920) and the rest on the lines of Swingle (1928 and 1931).

*Test 2.*—The solutions which did not show blue colour with potassium iodide-iodine solution (the test microtube of salivary gland of the larva and prepupa, of the midgut of larva, prepupa and pupa upto the age of 3 days) were further tested for the presence of maltase (the decomposition product) by the picramic acid test. Four drops of the incubated solution, 1 drop of 10% NaOH solution and 2 drops of a saturated aqueous solution of picric acid were placed in a microtube in the order mentioned. The tube was then placed in an electric oven at 60° C.

*Observation.*—The colour of the yellow picric acid was changed to reddish brown picramic acid.

*Inference.*—Maltose is formed as a result of hydrolysis of starch by the amylase present in the salivary gland of the larva and prepupa and of the midgut of larva, prepupa and pupæ upto the age of 3 days.

*Test 3.*—For further confirmation, Fehling's test and Fluckiger's test for sugar were performed.

*Fehling's test.*—A few drops of Fehling's solution No. 1 were taken in a test-tube and added to Fehling's solution No. 2 drop by drop till the precipitate that appears in the beginning disappeared. To a few drops of the mixed Fehling's solution 2 drops of the incubated solution, which had been tested for the presence of amylase, were added separately and heated and allowed to stand.

*Observation.*—Within 5 minutes of standing after heating, reddish brown precipitate of copper appeared.

*Inference.*—Sugar present.

*Fluckiger's test.*—Further confirmation was made by Fluckiger's test. Mixed a drop of 20% NaOH with an equal quantity of powdered copper tartrate upon a slide until copper was dissolved. Added a drop of incubated starch solution and heated gently.

*Observation.*—Red precipitate of copper appeared.

*Inference.*—Reducing sugar present. Thus the presence of amylase was finally confirmed in the salivary gland of the larva and prepupa and in the midgut of larva, prepupa and pupa upto the age of 3 days.

*(b) Detection of the presence of maltase*

*Test 1.*—Incubated a 15% maltose solution with the tissue suspensions of different parts of the gut of the larva, prepupa, pupæ of different ages and adult. After 46 hours, or more of incubation, tested a drop for the presence of glucose by osazone test. Ground 1 gm. of phenyl hydrazine hydrochloride



with 10 c.c. of glycerine till dissolved and filtered through glass-wool. Dissolved 1 gm. of sodium acetate in 10 c.c. of glycerine. Mixed a drop of phenyl hydrazine hydrochloride solution with a drop of sodium acetate upon a slide and added a drop of solution to be tested under a cover glass. Heated the slide upon a water-bath at 100° C. for 15 minutes and allowed to cool.

*Observation.*—An hour after cooling glucose osazone appeared in the incubated tissue suspension of the midgut of larva, prepupa and pupæ upto the age of 3 days and not in the rest.

*Inference.*—Maltose has been hydrolysed by maltase to glucose, maltase present.

*Test 3.*—Barfoed's test for monosaccharides was performed. A small quantity of acetic acid was added to a solution of copper acetate which is used to restrain the action of disaccharide. (If solution be too alkaline disaccharide may be hydrolyzed to glucose and the presence of glucose can possibly be detected even in the absence of maltase, hence the mixture of acetic acid and copper acetate is tested with a solution of maltose to see if maltose is hydrolysed to glucose or not.)

*Observation.*—A reddish brown precipitate appeared on heating in the midgut extracts of the larva, prepupa and pupæ upto the age of 3 days.

*Inference.*—Maltase present.

*(c) Detection of the presence of invertase*

*Test 1.*—Incubated a 15% sucrose solution with the tissue suspensions of gut. After incubating for 48 hours or more, tested a drop for the presence of fructose and glucose by osazone test and for reducing sugars by Fluckiger's test.

*Observation.*—Fructose osazone appeared immediately and glucose-osazone upon cooling, in the midgut extracts of the larva, prepupa, pupæ upto the age of 3 days, late pupa and the adult.

*Inference.*—Invertase present in the midgut of the larva, prepupa, pupæ upto the age of 3 days, late pupa and adult.

*(d) Detection of the presence of lactase*

*Test.*—Incubated a few drops of 15% lactose solution with the tissue suspensions of gut for 90 hours. Tested the material after incubation, for glucose, by osazone test.

*Observation.*—Glucose osazone appeared an hour after cooling in the midgut extract of the larva, prepupa and pupæ upto the age of 3 days.

*Inference.*—Lactase present.

*Alternative test.*—A drop of 15% lactose solution and 2 drops of glycerine extract were placed in a microtube and filled to the top with toluene and incubated for 72 hours at room temperature and tested for glucose by osazone test.

*Observation.*—Glucose osazone appeared in the same midgut extracts as tested by the first test.

*Inference.*—Lactase present.

(e) *Detection of the presence of lipase*

*Test.*—Lipase was also tested by using condensed milk instead of olive oil Acacia emulsion. Two drops of bromothymol blue were added to 25 c.c. of 10% solution of condensed milk and also powdered  $\text{NaHCO}_3$  or 1% NaOH solution till the solution turned light blue. One c.c. of blue milk solution and 2 drops of the extract were placed in a microtube and the rest of the tube filled with toluene as usual and incubated for 24–48 hours at room temperature.

*Observation.*—After 48 hours the blue colour changed to yellow.

*Inference.*—Lipase present.

(f) *Detection of the presence of protease*

*Test.*—The white of hen's egg was taken in a test-tube and drawn in fine capillary tubing; the 2 ends of the tube were sealed by heating in a flame and then it was transferred to a water-bath and heated slowly to coagulate albumen without air bubbles. The tubing, on cooling, was cut into pieces of about 1 cm. each and a piece immersed in gut extracts. The pH of the extracts was adjusted to 8 to make the medium alkaline and to 6.4 to make the medium acidic, by buffer solutions.

*Observation.*—The digestion of albumen was readily visible in the midgut extracts as in case of lactase and lipase.

*Inference.*—Protease present.

#### DISCUSSION

(i) *Feeding Habits.*—The study of the feeding habits of the larva and adult of *Papilio demoleus* reveals that like other lepidopterans, the nature of food and feeding habits of the larva differs to a very great extent from that of the adult. While the larva feeds voraciously on the leaves, the adult only

sucks the juice from the flowers. In the case of larvæ, 3 factors, *i.e.*, odour, taste and age of the plant influence the selection of food whereas in the case of adult, age does not play any role. Any *Citrus* plant at the flowering stage can attract adults of both the sexes. The restriction of this insect to *Citrus* alone proves the importance of odour and the restriction mainly to orange proves the importance of taste, whereas the restriction (of larvæ) mainly to nurseries proves the importance of the age of the plant, but in case of adults age of the host plant does not have any importance.

(ii) *Hydrogen-ion concentration*.—In insects in general the pH of different parts of the gut particularly the midgut usually varies slightly from neutrality, but there are cases of lepidopterous and trichopterous larvæ where the pH varies from weakly alkaline to strongly alkaline (Shinoda, 1930). Waterhouse (1949) working on 2 species of carnivorous lepidopterous larvæ and 40 species of adult Lepidoptera and on the basis of literature for the phytophagous, wool-eating and wax-eating lepidopterous larvæ generalises that the midgut alkalinity is characteristic of Lepidoptera. In the larvæ of *Papilio demoleus* the pH of the foregut, midgut, food of midgut and hindgut is 9.5, 8.8–9, 9.3 and 6.4–6.6 respectively. This goes to show that in the larvæ the digestive enzymes are active in an alkaline and strongly alkaline medium. The alkalinity of the foregut is difficult to explain, for the digestion does not take place in that region. The only plausible explanation appears to be that in order to ensure a certain pH for the midgut alkalinity the pH has to be maintained in advance.

The effect of pH of food on the pH of gut is still a controversial issue. Swingle's (1931) experiments on *Popillia japonica* larvæ show that the pH of the soil in which the larvæ were bred did not affect the pH of the gut and the mixture within the gut had a fairly constant pH. Waterhouse (1949) also working on Lepidoptera concludes that the midgut alkalinity of Lepidoptera is not dependent upon the feeding habits. The pH of different *Citrus* leaves varies from 5.4–6.2, whereas the pH of food content of midgut and of the midgut itself are 9.3 and 8.8–9 respectively. Thus the food is acidic, but the food content of the midgut and midgut itself are strongly alkaline. This goes to show that the food does not affect pH of the gut. The case of insects feeding on host plants of different families is likely to yield interesting result in this connection.

(iii) *Detection of enzymes*.—The qualitative test of enzymes has revealed that the salivary gland of the larva and prepupa contains a single enzyme amylase, but the salivary gland of the adult is devoid of enzymes. Wigglesworth (1950) also has noted that in the secretion of salivary gland of insects

either amylase is present or no enzyme at all. As the secretion of the midgut of the adult also has been noted to be devoid of amylase, it is clear that the adult does not utilise starch. Ulmann (1932) has demonstrated that the intestinal juices of a number of phytophagous insects are unable to digest starch granules, but can quickly hydrolyse boiled soluble starch which also is only partly utilized. Crowell (1946) has noted that lepidopterous larvæ do not utilize starch in spite of the presence of amylase in the salivary gland and midgut both. For this purpose, besides testing the salivary gland and midgut for amylase, the food of the midgut also was tested. Starch gave positive test showing thereby that amylase is at least secreted and as it is secreted it is very unlikely that starch in the midgut remains unacted. The midgut of the larva, besides secreting amylase, also secretes maltase, invertase, lactase, lipase and protease. The presence of amylase, maltase, invertase and lactase indicates that the larva can utilize carbohydrates. The presence of lipase shows that it needs fat also for its nutrition. Brown (1937) has noted complete absence of protein from the excreta showing thereby that it has been utilized by the insect. Bramstedt (1948) and Hinmann (1933) also have reached the conclusion that in the leaf-eating insects, protein is the chief dietary factor and is completely utilized along with soluble sugar. The presence of protease in the midgut of this insect goes to show that like other phytophagous insects it also needs protein for its use.

The reduction of enzymes from 6 in the larva to 1 in the adult is an interesting feature. All the larval midgut enzymes can be detected in the prepupa and pupa upto the age of 3 days only. The late pupa shows the presence of only one enzyme, *i.e.*, invertase which is present in the adult as well. Thus it appears that when the larval gut is replaced by the adult midgut the change in enzymes also takes place. The presence of invertase in the late pupa can be accounted for by the formation of adult gut at that stage.

There are distinct reasons for the change in the number of enzymes from 6 in the larva to 1 in the adult. There is great difference in the food and feeding habit of the larva and adult. The larva is a leaf-eater whereas the adult is only nectar feeder. Nectar contains sucrose and glucose alone, hence in order to convert sucrose into glucose and fructose, invertase alone is necessary. Thus it is clear that the change in food and feeding habit necessarily involves change in enzymes—even in different forms of the same insect, as shown by the present study.

#### SUMMARY

1. *Papilio demoleus* is a pest of the family Rutaceæ more particularly of *Citrus* sp. mainly in nurseries and the larvæ show special preference for

orange leaves. In the matter of selection of food, odour, taste and age of the plant play important roles in the case of larvæ and odour and taste alone in case of adults.

2. The hydrogen-ion concentration of the foregut of the larva, prepupa, 1 day old pupa, 2 days old pupa, 3 days old pupa, late pupa and adult are 9.5, 6.8, 6.4, 6, 6.8, 6.2 and 6 respectively, of midgut 8.8-9, 7, 6.4, 6.6, 6.8, 6.2-6.4 and 6.2-6.8 respectively and of hindgut 6.4-6.6, 7, 6.4, 6.6, 6.8, 6.2 and 6 respectively.

3. The foregut and hindgut of the larva, prepupa, pupæ and adult do not secrete any enzymes. The salivary gland of larva, prepupa and adult secrete amylase. The midgut of larva prepupa and pupa upto the age of 3 days secrete amylase, maltase, invertase, lactase, lipase and protease. The midgut of late pupa and adult both secrete only invertase. The proteases act in a slightly acidic media.

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\* Not seen in original.

# PARASITES OF *CHILO ZONELLUS* SWINHOE

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THE larvæ of *Chilo zonellus* S., a major pest of maize (*Zea mays*), juar (*Sorghum vulgare*) and sugarcane (*Saccharum officinarum*), cause damages to the stalks by boring through them. Of all the methods devised to control it, the best is biological control. The importance of its parasites is, therefore, evident. The author studied the parasites of *Chilo* in U.P. and recorded the following species for the first time.

TABLE I

Sl. No.	Order	Family	Name of the Parasite
1	Hymenoptera	Ichneumonidæ	<i>Xanthopimpla nursei</i> , Cam.
2	"	Braconidæ	<i>Stenobracon deesæ</i> , Cam.
3	"	"	<i>Apanteles</i> species
4	"	"	<i>Bracon chilonis</i> , Vier.

## 1. *Xanthopimpla nursei*, Cam.

The parasite was first recorded by Fletcher *et al.* (1919) at Nagpur and later by Ayyar (1923) from South India, but they have not studied its life-history. It has not been reported from anywhere else in India and abroad. At Kanpur the wasp emerges from the pupa of the host. One pupa of the parasite is present inside each pupa of the host. The longevity of the adult is 4.6 days as shown in the following table:—

TABLE II

## Longevity of *Xanthopimpla nursei*

Sl. No.	Emerged on	Died on	Longevity in days	Remarks
1	14-9-1952	18-9-1952	4	Female
2	9-10-1952	12-10-1952	3	
3	10-10-1952	17-10-1952	7	
4	15-10-1952	21-10-1952	6	
5	23-10-1952	26-10-1952	3	
		Total	..	23
		Average	..	4.6

2. *Stenobracon (Glyptomorpha) deesæ*, Cam.

Its distribution is restricted to India. It has been firstly reported by Ayyar (1923) from South India and later on it has been found and studied by Husain and Mathur (1923), Cherian and Israel (1938), Isaac (1936-37), Rahman (1941) and Narayanan *et al.* (1952) at Punjab, Coimbatore and Delhi, etc. It is an ectoparasite of the caterpillars. The larvæ of the parasite pupate inside cocoons in the galleries made by the hosts. Usually the females are bigger than the males in size, but the size of the parasites depends to a very great extent upon the size of the larvæ fed upon.

The longevity of the starved adults generally varies from 3-11 days except during winter when it may be about 28 days and the average longevity is 8 days. However, when fed on honey (Cherian and Israel, 1938) and glucose (Narayanan, 1948-49), the average longevity is reported to be 54 and 56 days respectively, showing the importance of food on longevity. The average prepupal and pupal periods, as noted by the author, are 4 and 10 days respectively.

TABLE III

*The prepupal and pupal periods and longevity of Stenobracon deesæ Cam.*

Sl. No.	Stopped feeding on	Pupated on	Prepupal period in days	Emerged on	Pupal period in days	Died on	Longevity in days
1	17- 9-1952	19- 9-1952	2	26- 9-1952	7	6-10-1952	10
2	18- 9-1952	21- 9-1952	3	6-10-1952	15	10-10-1952	4
3	4-10-1952	6-10-1952	2	13-10-1952	7	18-10-1952	5
4	5-10-1952	8-10-1952	3	15-10-1952	7	19-10-1952	4
5	6-10-1952	10-10-1952	4	19-10-1952	9	15-10-1952	10
6	8-10-1952	13-10-1952	5	19-10-1953	6	29-10-1952	10
7	8-10-1952	14-10-1952	6	22-10-1952	8	25-10-1952	3
8	8-10-1952	12-10-1952	4	22-10-1952	10	30-10-1952	8
9	8-10-1952	13-10-1952	5	28-10-1952	15	9-11-1952	11
10	18-10-1952	21-10-1952	3	26-10-1952	5	31-10-1952	5
11	18-10-1952	26-10-1952	8	20-11-1952	25	15-12-1952	25
Total			.. 45		114		91
Average			.. 4		10		8



3. *Apanteles* sp.

Two species of *Apanteles* are known to attack several species of the larvæ of *Chilo*. The species reported from India by Wilkinson (1928), Watanabe (1932), Pruthi (1939-40) and Bhatnagar (1948) is *Apanteles flavipes*, Cam. which is a cosmopolitan one. Its life-history has also not been studied in India.

About 20 larvæ of this parasite feed inside a single caterpillar, but come out of them when they are fully fed. After about 4-8 hours of the emergence from the host they pupate inside cocoons. These cocoons are in clusters surrounding the paralysed caterpillar. The host is not killed previously and in majority of the cases the caterpillars survive even when all the larvæ of the parasite are full-fed and emerge from them, but the vigour of the caterpillars is reduced and although they may feed alive for some days after the emergence of parasite larvæ, they do not pupate and ultimately die. The average prepupal and pupal periods and longevity, as shown in the following table are 3, 8 and 3 days respectively.

TABLE IV

Sl. No.	Larvæ came out of the caterpillar on	Pupated on	Prepupal period in days	Emerged on	Pupal period in days	Died on	Longevity in days
1	12- 9-1952	14- 9-1952	2	19- 9-1952	5	21- 9-1952	2
2	12- 9-1952	15- 9-1952	3	19- 9-1952	4	23- 9-1952	4
3	12- 9-1952	15- 9-1952	3	18- 9-1952	3	21- 9-1952	3
4	12- 9-1952	13- 9-1952	1	17- 9-1952	4	18- 9-1952	1
5	10-10-1952	10-10-1952	$\frac{1}{2}$	15-10-1952	5	17-10-1952	2
6	10-10-1952	12-10-1952	2	19-10-1952	7	21-10-1952	2
7	10-10-1952	13-10-1952	3	19-10-1952	6	22-10-1952	3
8	26-10-1952	27-10-1952	1	5-11-1952	9	9-11-1952	4
9	27-11-1952	30-11-1952	3	8-12-1952	8	13-12-1952	5
10	1-12-1952	5-12-1952	4	22-12-1952	17	27-12-1952	5
11	1-12-1952	5-12-1952	4	23-12-1952	18	26-12-1952	3
12	1-12-1952	6-12-1952	5	26-12-1952	20	31-12-1952	5
Total			..	32	106		39
Average			..	3	8		3

4. *Bracon chilonis*, Vier. (*Microbracon chilocida* Vier.)

According to Hadden (1930) it is native of Formosa; from there it was introduced in Hawaii on several occasions to control *Chilo* and other sugarcane borers. In India Ayyar (1927-28) has recorded it for the first time at Coimbatore and called it synonym of *Amyosoma (Microbracon) chelonis*, Vier. from Formosa. Later on Cherian and Narayanaswamy (1942) studied its biology in detail.

At Kanpur it has been observed as an ectoparasite. Its larvæ are similar in colour and general structure to, but smaller and lesser robust than the larvæ of *Stenobracon deesæ*, Cam. Generally, only about 2 larvæ have been noted on one paralysed and dead caterpillar. The pupæ are in the form of cocoons inside the tunnels of the plant made by the host and are generally stained dirty by the faeces and other contents of the host galleries, though some cocoons have been found pure white. The shapes of the cocoons are according to the space of the galleries in which they are found.

The pupal period and longevity on the average are 9 and 4.5 days respectively (Table V). The females lived longer than the males. According to Cherian and Narayanaswamy (1942) the pupal period ranges from 5-7 days, but in this case the range is 8-10 days. According to them the male and female adults survive 14 and 16 days and maximum of 59 and 55 days respectively, whereas at Kanpur the average longevity of an adult is only 4.5 days. So the conclusion is that the pupal period is longer and longevity is shorter at Kanpur than at Madras. The longer pupal period shows that the development at Kanpur is slower than in South. This variation may be due to climatic influence of the so-far situated places. But the longevity is very short at Kanpur in comparison to Madras. This fact can be explained that the adults at Kanpur have passed through their lives without food whereas at Madras parasites have been fed upon sugar solution.

TABLE V

*Showing the pupal period and longevity of Bracon chilonis, Vier.*

Sl. No.	Pupated on	Emerged on	Pupal period in days	Died on	Longevity in days	Remarks
1	9- 9-1952	17 -9-1952	8	21- 9-1952	4	Female
2	9- 9-1952	18 -9-1952	9	21- 9-1952	3	Male
3	29-10-1952	9-11-1952	10	16-11-1952	7	Female
4	1-11-1952	10-11-1952	9	14-11-1952	4	Male
Total			36		18	
Average			9		4.5	

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